Lung Cancer and Indoor Air Pollution in Xuan Wei, China

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In Xuan Wei County, Yunnan Province, lung cancer mortality is among China's highest and, especially in females, is more closely associated with indoor burning of "smoky" coal, as opposed to wood or "smokeless" coal, than with tobacco smoking. Indoor air samples were collected during the burning of all three fuels. In contrast to wood and smokeless coal emissions, smoky coal emission has high concentrations of submicron particles containing mutagenic organics, especially in aromatic and polar fractions. These studies suggested an etiologic link between domestic smoky coal burning and lung cancer in Xuan Wei.

XUAN WEI COUNTY, IN YUNNAN Province, China, has a population of about 1 million people, more than 90% of whom are farmers. Local industries include coal mining, electric power generation, and light manufacturing. The county has 20 communes with populations of 30,000 to 60,000. Tobacco smoking is common in males (40% or more), but rare in females (less than 0.1%). Local residents have traditionally used one of three major fuel types—"smoky" coal, "smokeless" coal, or wood—for domestic cooking and heating. The distribution of mines supplying smoky and smokeless coal is shown in Fig. 1. Fuel burning in shallow unvented pits has resulted in high indoor air pollution levels. Women customarily start the fire and cook; men generally spend most daylight hours outside the home.

The local medical care system of barefoot doctors, clinics, and hospitals ensures thorough lung cancer detection throughout the county. (In this report, "lung cancer" encompasses carcinomas of lung parenchyma, bronchus, and trachea.) Age-adjusted and unadjusted lung cancer mortality rates in China, Yunnan Province, and Xuan Wei are shown in Table 1 (1). Annual age-adjusted rates in Xuan Wei from 1973 to 1979 were 27.7 in males, among China's highest, and 25.3 in females, China's highest (2). The similarity between male and female lung cancer rates was unusual. In Xuan Wei, lung cancer was the only surveyed cancer for which mortality exceeded the national average (1). In the late 1970's, a clinical survey was also conducted to confirm the high lung cancer rates in Xuan Wei. In a surveyed population of 91,187 people, 262 lung cancer cases (287/100,000) were detected. Within this population, detection rates in farmers, office workers, and coal miners were 339, 118, and 15 per 100,000, respectively. This observation suggests that Xuan Wei lung cancer is not associated with non-agricultural occupations.

Unadjusted annual lung cancer mortality rates vary greatly across Xuan Wei communes (Fig. 1). Rates are generally highest in the central communes, especially Cheng Guan (CG), Lai Bin (LB), and Rong Cheng (RC), where smoky coal from LB is burned in more than 80% of homes. Between 1973 and 1975, the average of mortalities in communes with smoky coal mines (including RC and CG) was 34.7 per 100,000, as compared to 4.1 in communes without smoky coal mines. In 1982, a survey of past fuel use was conducted in more than 90% of homes in 11 Xuan Wei communes. As shown in Table 2, the commune-specific lung cancer mortality (1973–1975) was highly related to the commune-specific percentage of homes using smoky coal before 1958.

Thus, lung cancer mortality in Xuan Wei is associated with the domestic use of smoky coal. This association is especially strong in women, since they rarely use tobacco. At the same time, the wide variation in mortality across communes with smoky coal mines leaves open the possibility that specific environmental determinants of lung cancer may be more geographically restricted than is the broad entity "smoky coal."

To evaluate further the relation between domestic fuel and lung cancer in Xuan Wei, indoor air was characterized in CG and LB communes, where mortality is high and smoky coal is the predominant fuel, and in Re Shui (RS) commune, where mortality is low and wood (67%) and smokeless coal (33%) are the fuels used (Fig. 1). In each commune, indoor air was sampled in the fall of 1983 in four homes (typical in size, structure, and fuel burning for each commune) during fuel burning. Lung cancer had occurred in one each of the CG and LB homes. Smoky coal was burned in all sampled CG and LB homes; wood was burned in all sampled RS homes. Because of logistic difficulty of sampling RS homes in which smokeless coal was used, smokeless coal was transported from RS and burned in one CG
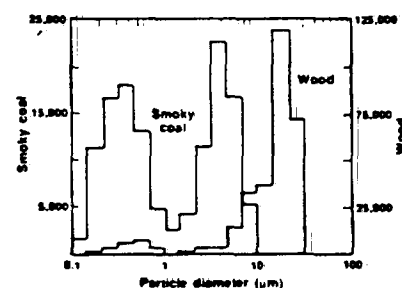

Fig. 2. Particle size distributions of indoor air particles collected during the combustion of smoky coal and wood. The y axis represents the particle volume (in cubic micrometers) within specified particle diameter range.

home. In the spring of 1984, four homes in RS burning smokeless coal routinely were sampled to confirm the previous study. High-volume (HV) samplers (flow rate 1.2 m³/min), with size-selective inlets (SSI), collected particulate matter smaller than 10 μm in diameter (PM10) on filters. Samplers were located 1.5 m from the fire and operated during three daily cooking periods (two cooking periods in LB) for 2 days, averaging 4 hours per day. At one CG home, a medium-volume (MV) sampler (flow rate 0.11
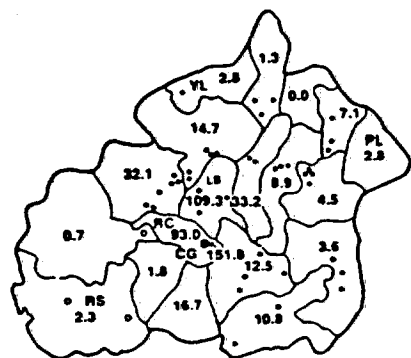
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Fig. 1. Map of Xuan Wei County, showing commune boundaries, each commune's unadjusted annual lung cancer mortality rate per 100,000 (both sexes, 1973–75), and mines supplying domestic coal: (●) smoky coal and (○) smokeless coal. Designated communes: CG, Cheng Guan; LB, Lai Bin; PL, Pu Li; RS, Re Shui; RC, Rong Cheng; and YL, Yang Liu.

Table 1. Annual lung cancer mortality rates in China and the United States.

Place	Time period	Mortality rate (per 100,000)						
		Unadjusted			Age-adjusted to 1964 China population		Age-adjusted to 1970 U.S. population	
		Males	Females	Combined	Males	Females	Males	Females
China	1973–75			5.0	6.8	3.2	12.3	5.7
United States	1970	53.7	12.0		30.0	6.3	53.7	12.0
Yunnan Province	1973–75			2.8	4.3	1.5	6.9	2.5
Xuan Wei County	1973–79	27.0	24.5		27.7	25.3	43.2	38.7
Three high-mortality Xuan Wei communes (Cheng Guan, Lai Bin, Rong Cheng)	1973–79	114.4	120.6		118.0	125.6	186.8	193.4
55- to 59-year age group in three high-mortality communes	1973–79	849.4	904.0					
Three low-mortality Xuan Wei communes (Pu Li, Re Shui, Yang Liu)	1973–79	4.0	2.8		4.3	3.1	5.8	4.3

m^3/min) with a PM10 SSI, a filter, and an XAD-2 resin trap collected particles and semivolatile organics. Each of the three fuel types was burned 6 hours per day for 2 days. During smoky coal and wood burning, a personal sampler (flow rate 1 liter/min) collected particles for physical characterization, including sizing by scanning-electron microscopy and elemental analysis by x-ray fluorescence analysis. The fuel samples of smoky and smokeless coal from mines in LB and RS, respectively, were analyzed according to the methods of the American Society for Testing and Materials (ASTM) (3).

Organic material was removed from the filters by Soxhlet extraction and XAD-2 with dichloromethane. The filter extracts were then fractionated into aliphatics, aromatics, moderately polar, and polar components by silica gel fractionation (4). Extracts and fractions were analyzed with a Finnigan 4500 gas chromatography/mass spectrometry (GC/MS) (5). With the MS in the multiple ion detection mode, the extracts were quantitatively analyzed for 26 compounds, including the polycyclic aromatic hydrocarbons (PAH) and nitrogen heterocyclics that have shown animal carcinogenicity (6). To identify major components, the extracts were further analyzed with the MS in the full-scan detection mode. The standard Salmonella typhimurium plate incorporation assay with minor modifications was used to assess the mutagenicity of the organic extracts and fractions (7, 8). Samples were tested in strain T98 with and without metabolic activation by S9. The fractions showing the most mutagenicity were further analyzed with the MS in the full-scan detection mode.

The smoky coal contained 23.1% volatiles, 66.7% carbon, 23.5% ash, and 0.2% sulfur; its heating value was 27.1 MJ/kg. By ASTM classification (3), the smoky coal was ranked as a medium-volatile bituminous coal with low sulfur and high ash. Except for elevated levels of nickel (64 ppm), its trace elemental concentrations were comparable to those of analyzed U.S. anthracite and bituminous coals. The smokeless coal contained 13.8% volatiles, 38.5% carbon, 1.9% sulfur, and an extremely high level of ash, 49.3%; its heating value was low, 14.5 MJ/kg.

The concentrations of airborne particles (PM10) inside homes during smoky coal and wood combustion were very high [that is, 24.4 ± 3.3 (mean ± SEM), 9.5 ± 1.6, and 22.3 ± 2.0 mg/m^3 for CG, LB, and RS, respectively], whereas concentrations during combustion of smokeless coal were considerably lower [1.8 mg/m^3 for the CG home burning smokeless coal (1983) and 1.1 ± 0.1 mg for the four RS homes burning smokeless coal (1984)]. Figure 2 shows the size distributions of indoor air particles during combustion of smoky coal and wood. Both distributions were bimodal. Fifty-one percent of the particles from smoky coal combustion were less than 1 μm in diameter; the rest were between 1 and 10 μm. Most particles were spherical. In marked contrast, 94% of the particles from wood combustion ranged from 1 to 30 μm; only 6% were less than 1 μm in diameter. The x-ray fluorescence analysis showed that most of the particles collected during smoky coal combustion were not inorganic. This was confirmed by the solvent extraction studies, which showed a high percentage of organic mass in the particles from smoky coal combustion. The ratio of organics to soot to unburned fuel in the particles from smoky coal, smokeless coal, and wood combustion were 80:10:10, 10:30:60, and 10:40:50, respectively.

The samples from smoky coal combustion contained high concentrations of organic matter (72 to 82%) whereas the samples from the other two fuels, especially smokeless coal, contained much lower percentages (27% for smokeless coal sample; 55% for wood sample). Full-scan GC/MS analysis of the organic extracts of the smoky and smokeless coal combustion samples showed their major components to be PAH and methylated PAH, whereas the major components of the wood samples were hydroxylated aromatics, aliphatic aldehydes and alcohols, and PAH. Fewer PAH compounds were detected in the wood samples than in the coal samples. The results of the PAH analysis for selected carcinogenic compounds are shown in Fig. 3. The highest PAH levels were consistently found in the smoky coal samples from CG, followed by those from LB. The wood samples contained much lower levels of PAH, and the smokeless coal sample contained the lowest levels. The smoky coal samples also contained the highest levels of nitrogen heterocyclic compounds, for example, dibenz-[a,j]acridine: 688 ng/m^3 in the CG smoky

Table 2. Percentage of households burning smoky coal before 1958 and 1973–1975 unadjusted lung cancer mortality in 11 Xuan Wei communes.

Commune	Smoky coal (%)	Lung cancer mortality (per 100,000)
Cheng Guan	100.0	151.8
Lai Bin	89.7	109.3
Rong Cheng	81.9	93.0
Long Tan	78.0	32.1
Long Chang	76.1	33.2
Hai Dai	49.7	10.8
Pu Li	35.2	2.8
Ban Qiao	34.0	16.7
Luo Shui	2.7	1.8
Re Shui	0.0	2.3
Xi Ze	0.0	0.7

coal sample, 7 ng/m³ in the smokeless coal sample, and below the detectable limit in the wood sample. Filters in both the HV and MV samplers collected a wide range of PAH compounds (three to seven rings), whereas the XAD collected mostly compounds of four or fewer rings. The coal extracts contained a higher percentage of aromatic compounds (36 to 40%) than the wood sample extracts (7%). Most (76%) of the mass in the wood extract was in the polar fraction.

Most samples required metabolic activation to display significant mutagenicity (Table 3), which also suggested the presence of PAH. The CG smoky coal samples showed the highest mutagenic activity, followed by the LB smoky coal samples. The wood samples displayed lower activity, and the smokeless coal samples displayed the least. The aromatic and polar fractions of the smoky coal samples contributed most of the mutagenic activity. The aliphatic fractions (accounting for 1 to 5% of organic mass in the extracts) of all samples were nonmutagenic. All XAD samples displayed little mutagenicity.

The fractions that displayed the most mutagenic activity—the aromatic and polar fractions of the smoky coal samples—were subjected to GC/MS analysis to identify major components. The aromatic fractions consisted primarily of PAH, methylated PAH, and nitrogen heterocyclic compounds. The polar fractions of the smoky coal samples included nitrogen- and oxygen-containing compounds.

This study revealed that the residents of CG, where lung cancer rates are highest and the fuel is smoky coal, are exposed to very high indoor particulate concentrations [more than 100 times the proposed U.S. ambient air 24-hour standard for PM10 (9)]. The particles from smoky coal combustion were mostly organic and submicron—a size that stays longer in the air and can be effectively deposited in the lung after inhalation (10)—and contained high levels of carcinogenic PAH compounds. Indoor ben-

Fig. 3. Indoor polycyclic aromatic hydrocarbon concentrations by HV sampling (PM10). Values are mean (± SEM, where shown) of the compounds, showing sufficient evidence of carcinogenicity in animals (6). BA, benz[a]anthracene; 5MC, 5-methylchrysene; BFT, benzofluoranthenes; B(a)P, benzo[a]pyrene; IDP, indeno[1,2,3-cd]pyrene; and DBP, dibenzo[a,e]pyrene. Each bar represents data from four homes of each commune, except the bar for smokeless coal. Smokeless coal data were from one home.

zo[a]pyrene concentrations during cooking are comparable to occupational exposure levels, such as those in coke oven plants (11). Mutagenicity results were also consistent with the epidemiologic findings: the sample from CG showed the most mutagenic activity, followed by the sample from LB, the commune with the next highest lung cancer mortality; the samples from RS, the low-mortality commune, showed lower mutagenic activity.

The differences, based on per cubic meter of air (Fig. 3 and Table 3), in PAH concentrations and mutagenicity of the CG and the LB homes were mainly due to the difference in the particulate concentrations during cooking (CG homes had 1.5 times higher particulate concentrations than LB homes). Based on per microgram of the organic mass extracted from the particles, the PAH and mutagenicity of samples from homes in these two communes were similar (2.6 and 3.3 revertants per microgram for CG and LB neat organic extracts, respectively, in contrast to 0.7 revertant per microgram for the RS sample). Differences in particulate concentration were mainly due to differences in cooking habits. The LB residents customarily cook only a breakfast (with a longer period of a more steady fire) and a supper each day, whereas the CG (and RS) residents usually cook three meals with shorter periods that require more frequent starting and stoking of the fire, and hence
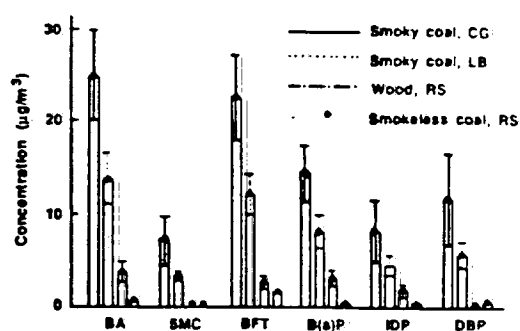
generate higher smoke emissions than a steady fire.

The aromatic fractions from coal combustion contained more methylated PAH compounds than the sample from wood combustion (for example, the ratios of 5-methylchrysene:chrysene were 0.27:1 in smoky coal, 0.10:1 in smokeless coal, and 0.04:1 in wood). Some methylated PAH compounds are known to be more carcinogenic than their parent PAH compounds (6). The high levels of methylated PAH compounds from smoky coal combustion may contribute to the high lung cancer rates in CG and LB. The presence of nitrogen heterocyclic compounds in the smoky coal combustion sample may also contribute to the high rates. We observed that, in addition to PAH, the polar compounds (accounting for 30% of the organic mass) also showed significant mutagenicity in the smoky coal samples.

This study, like other studies, suggested little association between domestic open-fire wood smoke and lung cancer (12). The less efficient lung deposition of the large particles from wood combustion plus the lower concentrations of biologically active compounds may both contribute to the low rate of lung cancer in RS, where wood is a common fuel. Smokeless coal, the other fuel used in RS, gave low indoor concentrations of particles of low organic content. Unlike the particles from smoky coal combustion, 90% of the particles from smokeless coal

Table 3. Mutagenicity of Xuan Wei indoor air particles (PM10). Data represent one home in each commune. The home selected was the one showing mutagenic activity in the neat sample close to the mean value of all four homes sampled in each commune.

| Fraction | Mutagenicity (revertants × 10³ per cubic meter)* | | | |
	Smoky coal, CG	Smoky coal, LB	Wood, RS	Smokeless coal, RS
Neat sample	58.9 ± 6.8	17.0 ± 1.5	11.1 ± 0.5	1.3 ± 0.05
Aliphatics (hexane)	0	0	0	0
Aromatics (hexane/benzene)	18.1 ± 1.6 (30.7)†	6.7 ± 0.4 (39.4)	3.1 ± 0.2 (27.9)	0.5 ± 0.03 (38.5)
Moderately polar compounds (dichloromethane)	8.7 ± 0.8 (14.8)	4.0 ± 0.3 (23.5)	4.4 ± 0.2 (39.6)	0.3 ± 0.02 (23.1)
Polar compounds (methanol)	24.4 ± 1.4 (41.4)	7.9 ± 0.3 (46.5)	5.0 ± 0.5 (45.0)	0.4 ± 0.03 (30.8)

*Results from high-volume sampling. The organic extracts of the particulate samples were solvent-exchanged to dimethyl sulfoxide and tested at a minimum of five doses in triplicate. A sample was considered mutagenic if a dose-response relation was observed. The slope of the initial linear portion of each dose-response curve was calculated by least-squares linear regression to obtain the number of revertants per microgram of organics. The number of revertants per cubic meter of air was calculated by multiplying the number of revertants per microgram of organics by the micrograms of organics per cubic meter of air. Standard errors of slopes are also shown. †Numbers in parentheses represent the percentage of the neat sample.

combustion were soot and unburned fuel. These may also explain the lower rate of lung cancer in R.S.

Thus, to date, the collaborative studies of Xuan Wei lung cancer have shown consistency among epidemiologic, physical, chemical, and toxicologic findings. The accumulating data increasingly suggest an etiologic link between indoor smoky coal burning and lung cancer.

Note added in proof: In our recent mouse skin tumor initiation promotion studies in female SENCAR mice using the organic extract as the tumor initiator and 12-O-tetradecanoylphorbol-13-acetate (TPA) as the promoter, the organic extract of the smoky coal sample induced more papillomas per mouse at the dose of 10 mg per mouse (7.5 papillomas after 26 weeks of promotion) than the extract of the wood sample (2.1 papillomas). When applied dermally twice weekly (total 2 mg per mouse per week) to the mice without TPA promotion,

the smoky coal sample induced carcinomas in 35% of the treated animals while no carcinomas were observed in the wood sample–treated mice or in the solvent control-treated mice after 52 weeks of the treatment *(13)*.

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14. This study was conducted under the China–U.S. Protocol, Annex 1, II.A. for Scientific and Technical Cooperation in the Field of Environmental Protection. We thank G. P. Qu and R. S. Cortesi for making the initiation of this project possible, the staff of the Institute of Health in Beijing, the Anti-Epidemic Stations of Yunnan Province, Qu Jing Region, and Xuan Wei County for excellent assistance in air sampling; and V. S. Houk, W. B. Riggan, J. L. Miller, and F. H. Haynie for technical assistance.

12 May 1986; accepted 14 October 1986

Preferred Microtubules for Vesicle Transport in Lobster Axons

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The hypothesis that transported vesicles are preferentially associated with a subclass of microtubules has been tested in lobster axons. A cold block was used to collect moving vesicles in these axons; this treatment caused the vesicles to accumulate in files along some of the microtubules. Quantitative analysis of the number of vesicles associated with microtubule segments indicated that lobster axons have two distinct populations of microtubules—transport microtubules that are the preferred substrates for vesicle transport and architectural microtubules that contribute to axonal structure.

MICROTUBULES ARE THE LINEAR substrates for vesicle transport in axons *(1–3)*; in addition to their role in vesicle transport, they are essential structures in axonal architecture *(4)*. These two functions—transport and architecture—place different demands upon the microtubules. In some cases, the requirements of architecture actually appear opposite to those of vesicle transport. For example, axonal microtubules have an extensive system of sidearms that contribute to the architecture of the axonal cytoskeleton by linking the microtubules to neighboring cytoskeletal structures *(5)*. Although such cross-links help to define axonal architecture, they offer potential resistance to the movement of transported vesicles along the microtubules *(6)*.

There and other differences between the requirements of vesicle transport and architecture may have fostered the evolution of two classes of microtubules—one class that is the preferential substrate for vesicle transport and another class that contributes primarily to axonal architecture. To examine the possibility that axons contain a subclass of microtubules that are the preferred substrates for vesicle transport, we analyzed the distribution of transported vesicles in lobster axons. In lobster axons, microtubules are the only long polymers *(7)*. These axons, and those of other arthropods, lack neurofilaments; arthropods may have lost the genes for neurofilament proteins when the arthropod lineage diverged from the other metazoan phyla *(4, 8)*. In arthropod axons, microtubules fulfill all of the architectural and transport functions required of cytoskeletal polymers in axons.

To determine whether transported vesicles moved preferentially along particular microtubules in lobster axons, we used the cold-block method to collect and distinguish transported vesicles. A small region of the axon is cooled to 2° to 4°C *(3, 9, 10)*. When moving vesicles reach the cold block, they stop and accumulate along the normal pathways of transport *(3, 9, 10)*. In vertebrate and molluscan axons (both of which contain neurofilaments), the transported vesicles accumulate in files along microtubule domains, which are surrounded by neurofilaments; these microtubule domains are the

Table 1. Vesicle distribution among lobster axon microtubules assuming one homogeneous population of microtubules. Total vesicles, 346; average vesicles per microtubule, 1.18.

Vesicles per microtubule	Microtubules (number)	
	Observed[a]	Poisson prediction ($m = 1.18$)
0	212	90
1	14	106
2	11	63
3	9	25
4	10	7
5	12	2
6	8	0
7	8	0
8	4	0
9+	5	0
Total	293	293

[a]Observed and expected values are statistically distinct by both the chi-square test and the Kolmogorov-Smirnov test ($P < 0.01$).

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